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**TITLE:**
Regulation and Action of SKP2 in Cell and Tumor Models: Mechanisms Underlying Aggressive Growth in Basel-Like Breast Cancer

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**13. SUPPLEMENTARY NOTES**

**14. ABSTRACT**

The objective of this research is to further our understanding of the molecular mechanisms underlying the aggressive growth of estrogen receptor (ER)-negative, basal-like breast tumors. My goal is to determine if SKP2 is a viable new therapeutic target to specifically treat patients who have tumors that are independent of ER signaling. The most significant finding during this research period is that SKP2 protein was expressed in 60% (21 of 35) of ER-negative tumors, 25% (26 of 104) of ER-positive tumors, and 10% (5 of 50) reductive mammoplasty tissues. These data suggest that SKP2 overxpression is a phenomenon associated with ER-negative tumors. With an additional 150-300 ER-negative tumor cases this relationship will be better elucidated.

**15. SUBJECT TERMS**
ER-negative, Breast Cancer, SKP2

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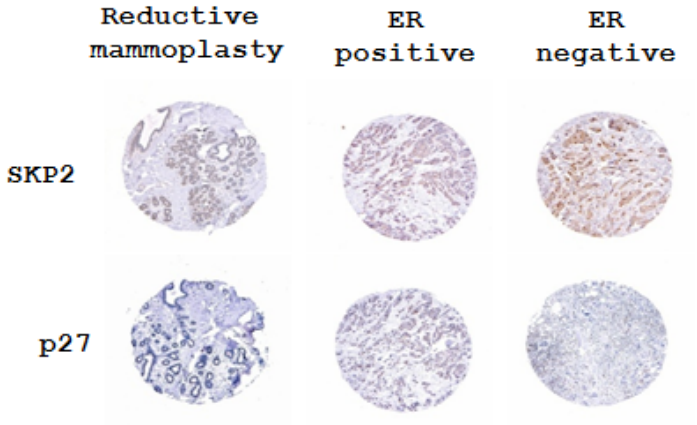
**Introduction:**

The objective of this research is to further our understanding of the cellular and molecular mechanisms underlying the aggressive growth of ER-negative, basal-like tumors. The goal is to identify new therapeutic targets to specifically treat patients that have tumors that are independent of ER signaling as these tumors are more often ER-negative. Past work from our lab and others has suggested that S-phase kinase-associated protein 2 (SKP2) plays an important role in breast tumorigenesis and would make a good therapeutic target. By utilizing three models (human tissue, animal models, and tissue culture) in which to characterize the role of SKP2 in breast cancer, we can obtain a better understanding of the molecular mechanisms underlying the aggressive tumor growth of basal-like breast tumors. It is anticipated that results from these studies will show that SKP2 would make a good therapeutic target for the treatment of women with basal-like tumors that are often associated with poor clinical outcome and tend to be ER-negative.

**Body:**

Task 1: During the first year of this three year study, I have obtained 189 formalin-fixed paraffin-embedded reductive mammoplasty and breast carcinoma tissue microarrays (TMA). These samples have been stained for SKP2, p27, phosphor-p27, CDK2, cyclin D1, and cyclin E. In collaboration with the Dr. Christopher Otis, SKP2 expression was examined in 35 ER-negative, 104 ER-positive, and 50 reductive mammoplasty tissue samples by immunohistochemisty. SKP2 protein was found to be highly expressed in 60% (21 of 35) of ER-negative tumors and 25% (26 of 104) of ER-positive tumors, and 10% (5 of 50) reductive mammoplasty tissues. Additionally, 44% (8 of 18) of ER-negative tumors expressed high SKP2 and low p27. Representative SKP2 and p27 stained punches are shown below (Figure 1).

**SKP2 PROTEIN EXPRESSION IS HIGH IN 60% OF ER-NEGATIVE BREAST CANCERS**




| | SKP2 positive | SKP2 negative | |
|---|---|---|---|
| ER-negative | 26 | 78 | 104 |
| ER-positive | 21 | 14 | 35 |
| Reductive mammoplasty | 5 | 45 | 50 |
| | 52 | 137 | |

Figure 1: SKP2 expression was examined in 35 ER-negative, 104 ER-positive, and 50 reductive mammoplasty tissue samples by immunohistochemisty. SKP2 protein was found to be highly expressed in 60% (21 of 35) of ER-negative tumors and 25% (26 of 104) of ER-positive tumors, and 10% (5 of 50) reductive mammoplasty tissues. Additionally, 44% (8 of 18) of ER-negative tumors expressed high SKP2 and low p27. Representative SKP2 and p27 stained punches are shown.

Task 2: I have obtained a SKP2 shRNA and negative control shRNA expression vector that also expressed Turbo GFP. Using this vector I have been able to establish a TMX2-28 cell line stably transfected with the negative control vector. Currently a stably transfected TMX2-28 cell line with the SKP2 shRNA construct is being established.

Task 3: To date, I have isolated RNA and protein from untransfected TMX2-28, MCF-7, and MDA-MB-231 cells. With the resulting RNA I have established a gene expression profile for SKP2 and its associated proteins in the aforementioned cell lines (Figure 2). I have also collected SKP2 knocked down TMX2-28 RNA and established a gene expression profile for SKP2 and its associated proteins when compared to untransfected cell lines (Figure 3).

**TMX2-28 CELLS OVEREXPRESS A NUMBER OF CELL CYCLE GENES ASSOCIATED WITH SKP2**

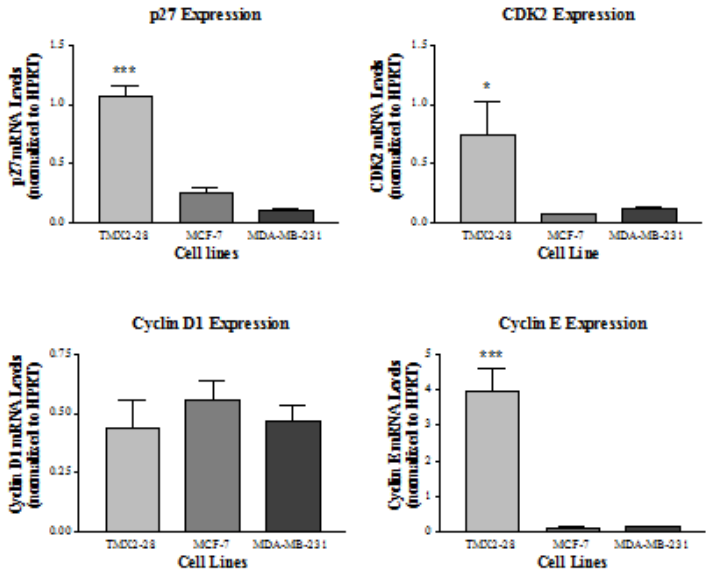



Figure 2: Gene expression was determined using real time qRT-PCR (One-way ANOVA, p27: $p=0.0326$; CDK2: $p<0.0001$; Cyclin E: $p<0.0001$)

**KNOCKDOWN OF SKP2 IN TMX2-28 CELLS DOES NOT RESULT IN SIGNIFICANT CHANGES IN THE GENE EXPRESSION OF THE CELL CYCLE GENES ASSOCIATED WITH SKP2**

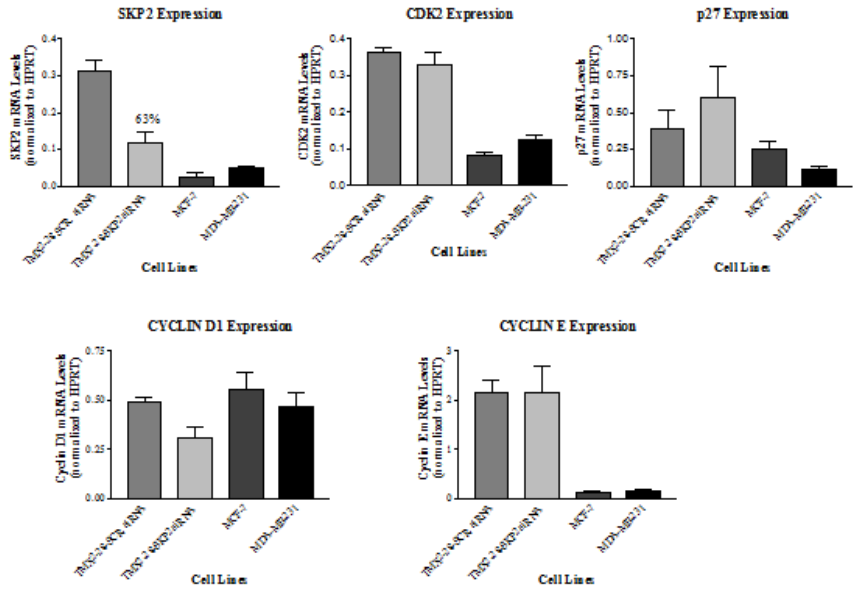



Figure 3: TMX2-28 cells were transiently transfected with siRNA targeting SKP2 or a scrambled (SCR.) version of the sequence (negative control) using a lipid based transfection agent. Forty-eight hours post transfection RNA was isolated and gene expression was determined using real time qRT-PCR (Unpaired T Test with Welch's correction)

**Key Research Accomplishments:**

Training Accomplishments:

- Continue collaborations with **Dr. Christopher Otis**, Director of Surgical Pathology at Baystate Medical Center; **Dr. Brian Pentecost**, New York Department of Health,; **Dr. Sallie Smith-Schneider**, Pioneer Valley Life Sciences Institute; and **Dr. Douglas Anderton**, Associate Dean for Research Affairs, Director of Social and Demographic Research Institute
- Current and active member of AACR, AAAS, and SACNAS
- Continue to talk and meet with my mentor Dr. Kathleen Arcaro on a daily basis
- Attend weekly cancer and chemoprevention journal club, molecular and cellular biology seminar and colloquia, animal biotechnology and biomedical science seminar, microbiology seminar
- Awarded Rays of Hope grant to fund pathology work

**Research accomplishments:**

- 189 tissue samples stained for SKP2 and its associated proteins
- Scored and analyzed SKP2 and p27 staining
- Obtained tissue microarrayer to construct additional TMAs
- Currently learning how to construct TMAs
- Obtained shRNA targeted against SKP2 and a negative control shRNA
- Transfected TMX2-28 cells with negative control shRNA and successfully established a stably transfected cell line
- Isolated RNA and protein from TMX2-28, MCF-7, and MDA-MB-231 cell lines
- Established gene expression profile for SKP2 and its associated proteins in TMX2-28, MCF-7, and MDA-MB-231 cell lines both untransfected and transiently transfected with siRNA targeted against SKP2 using quantitative real time RT-PCR
-

**Reportable Outcomes:**

As a result of my research thus far, I have established a negative control shRNA stably transfected TMX2-28 cell line. This work has also led to the acquirement of a Rays of Hope grant from Baystate Medical Center to fund the immunohistochemistry part of this project.

- Analysis of SKP2 and Associated Cell Cycle Proteins in Estrogen-Receptor Negative Breast Cancers (PI- Kathleen Arcaro, Co-investigators-Christopher Otis, Katerina Fagan-Solis, Douglas Anderton)

**Conclusion:**

The first year of this study has led to establishment of a cell line, the establishment of a TMX2-28 gene expression profile for SKP2 and its associated proteins, and the beginning of immunohistochemical protein expression analysis. Additionally, it has let to the establishment and continuation of my training through collaborations and interactions with a number of clinicians, pathologists, bench scientists and epidemiologists. In the second year of this study I expect to complete all immunohistochemical (task 1), cell line establishment (task 2), gene/protein expression (task 3), and begin *in vivo* work.

**References:** None

**Appendices:** None